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Parenteral aluminum administration in the dog: II. Induction of osteomalacia and effect on vitamin D metabolism

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Parenteral aluminum administration in the dog: II. Induction of osteomalacia and effect on vitamin D metabolism. There is an association between bone aluminum (Al) accumulation and dialysis-associated osteomalacia (OM). To study whether Al is pathogenic in OM, quantitative bone histomorphometry was done in six dogs before (Bx 1) and after (Bx 2) 3 to 5 weeks of intravenous Al administration (1 mg Al^{+++}/kg/day). Bone Al was determined by histochemical and chemical methods. The percent osteoid rose from 2.8 ± 0.8 to 7.0 ± 4.3% (mean ± SD), $P < 0.05$, and osteoid width increased from 5.7 ± 0.6 to 8.0 ± 1.2 μ, $P < 0.01$, after Al. Bone Al rose from 1.3 ± 1.6 to 94.0 ± 19.0 mg/kg after Al, and the severity of OM, expressed as either percent forming surface or percent osteoid, correlated with bone Al measured histochemically and expressed as either percent surface or percent area of trabecular bone staining for Al (r = 0.85 − 0.90, $P < 0.01$). Poor tetracycline uptake (six dogs), which indicates impaired mineralization, and little or no separation of tetracycline labels (four dogs) were noted at Bx 2; thus, bone apposition and formation rates were below the limits of detection. Resorptive surface did not change but trabecular volume, expressed as percent of tissue volume, fell from 22.1 ± 3.0 to 17.1 ± 1.4%, $P < 0.05$. Serum levels of $1,25(OH)_2D$ fell from 26.8 ± 9.1 to 4.5 ± 5.5 pg/ml after 17 days of Al; serum 25(OH)D levels were unchanged. These data indicate that Al can cause OM and that its severity correlates with the bone Al content. These findings suggest that Al has a direct effect on bone; the role of altered vitamin D metabolism in mediating these changes remains to be defined.

Administration parentérale d'aluminium chez le chien: II. Induction d'une ostéomalacie et effet sur le métabolisme de la vitamine D. Il existe une association entre l'accumulation d'aluminium osseux (Al) et l'ostéomalacie associée à l'hémodialyse. Afin d'étudier si Al a un rôle pathogène dans OM, une histomorphométrie osseuse quantitative a été effectuée chez six chiens avant (Bx 1) et après (Bx 2) 3 à 5 semaines d'administration intraveineuse d'Al (1 mg Al^{+++}/kg/jour). L'Al osseux a été déterminé par des méthodes histochimiques et chimiques. Le pourcentage d'ostéoïde s'est élevé de 2,8 ± 0,8 à 7,0 ± 4,3% (moyenne ± SD), $P < 0,05$, et l'épaisseur ostéoïde s'est crû de 5,7 ± 0,6 à 8,0 ± 1,2 μ, $P < 0,01$, après Al. L'Al osseux a augmenté de 1,3 ± 1,6 à 94,0 ± 19,0 mg/kg après Al, et la sévérité de l'OM, exprimée soit en pourcentage de surface en formation, soit en pourcentage d'ostéoïde, était corrélée avec l'Al osseux mesuré histochimiquement et exprimé soit en pourcentage de surface, soit en pourcentage d'aire d'os trabéculaire donnant une coloration pour l'Al (r = 0,85 − 0,90, $P < 0,01$). Une faible fixation de tétracycline (six chiens) ce qui indique une anomalie de minéralisation, ou une faible ou l'absence de séparation des marquages par la tétracycline (quartre chiens) ont été notées à Bx 2; ainsi, les vitesses d'apposition et de formation osseuses étaient en dessous des limites de détection. La surface de résorption n'a pas changé, mais le volume trabéculaire, exprimé en pourcentage du volume tissulaire, a diminué de 22,1 ± 3,0 à 17,1 ± 1,4%, $P < 0,05$. Les niveaux sériques de $1,25(OH)_2D$ ont baissé de 26,8 ± 9,1 à 4,5 ± 5,5 pg/ml après 17 jours d'Al; les concentrations sériques de 25(OH)D étaient inchangées. Ces données indiquent qu'Al peut entrainer une OM dont la sévérité est corrélée au contenu osseux en Al. Ces résultats suggèrent qu'Al a un effet direct sur l'os; le rôle de l'altération du métabolisme de la vitamine D dans ces modifications reste à déterminer.

A growing body of evidence suggests an association between the syndrome "dialysis-related osteomalacia" and the accumulation of aluminum in bone. Several clinical studies have indicated a high incidence of fracturing bone disease in patients undergoing hemodialysis with water containing aluminum [1–4]. A high bone aluminum content has been demonstrated by both chemical and histochemical techniques in many dialysis patients, a substantial portion of whom show histologic evidence of osteomalacia [5–9]. Furthermore, the severity of osteomalacia, as assessed by the degree of osteoid accumulation, correlates positively with the aluminum content of bone [6, 7, 10]. A recent report suggests that aluminum deposition in bone can occur early in the course of clinical dialysis-associated osteomalacia, perhaps antedating overt histological changes [8].

It is possible that the passive uptake of aluminum by poorly or unmineralized osteoid might account for the associations described. The kidney is the major route for the excretion of aluminum [11], and it is not unexpected that patients with markedly reduced renal function might retain aluminum. Indeed, bone is one of the primary tissues in which aluminum accumulates in the presence of renal insufficiency [12].

Few experimental studies have evaluated the potential for bone toxicity of aluminum or its relationship to the pathogene-

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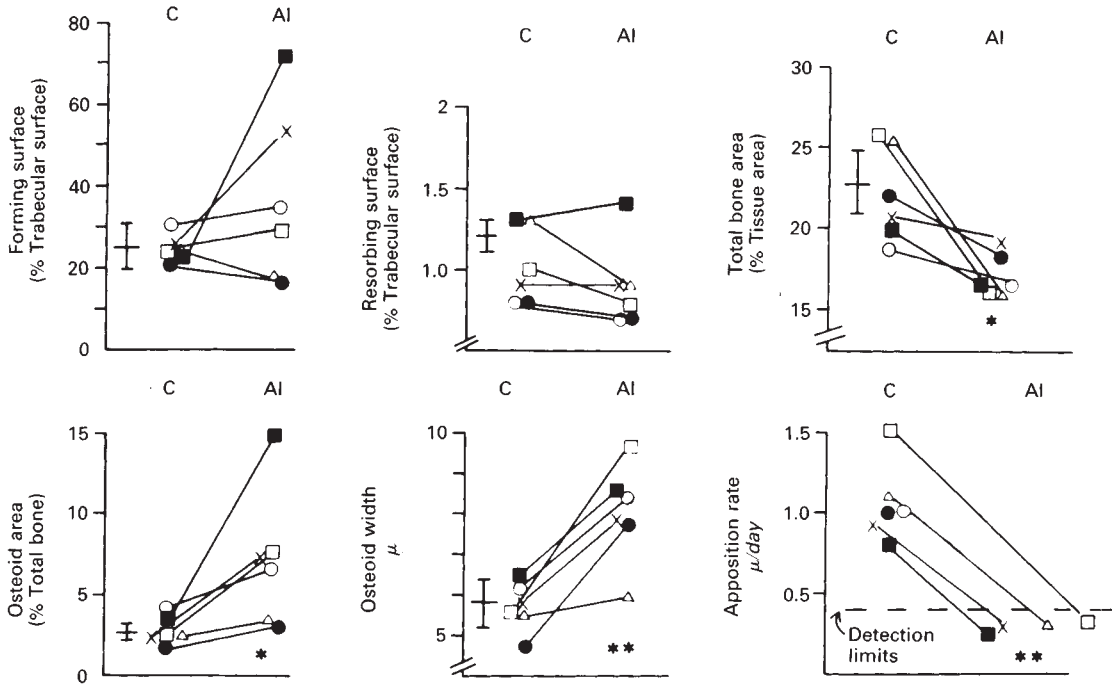

Fig. 1. *Quantitative histomorphometric data and dynamic histology in six dogs loaded with aluminum for three (dogs 1 and 2) or 5 weeks.* The brackets are the mean ± SD of normal values obtained in four separate female dogs. The apposition rates after aluminum loading were calculated only in the four dogs that received double tetracycline labeling before the second biopsy was performed. Abbreviations are: C, control biopsy; Al, after aluminum loading. Individual numbered dogs are represented by the following symbols: 1, ●; 2, ○; 3, X; 4, ■; 5, △; 6, □. The *asterisks* represent values that differ from control: *, $P < 0.05$; **, $P < 0.01$.

sis of osteomalacia. Ellis, McCarthy, and Herrington [9] described defective mineralization in the tibial epiphyses of rats given intraperitoneal aluminum injections for as long as 105 days. The mineralization of newly forming bone became normal after the administration of aluminum was stopped. Two other reports indicate that aluminum loading can induce osteomalacic changes in the trabecular bone of rats [13, 14]. In one study this occurred only in uremic animals [14], whereas in the other, both uremic and nonuremic rats were affected [13].

To investigate the effect of aluminum administration on bone, quantitative bone histomorphometry was done in dogs given daily parenteral aluminum injections for 3 to 5 weeks. In addition, serial serum levels of 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin-D [1,25$(OH)_2$D] were measured.

Methods

Six female mongrel dogs weighing 18 to 34 kg were studied. Animals were maintained on standard laboratory dog chow (Ralston Purina, St. Louis, Missouri) for the duration of study.

All dogs received bolus intravenous injections of aluminum chloride containing 1 mg/kg elemental aluminum 5 days each week. Four dogs received injections of aluminum for 5 weeks; the injections were terminated after 3 weeks in two dogs.

Serum levels of calcium, phosphorus, 25(OH)D, and 1,25$(OH)_2$D were measured by methods reported elsewhere [15, 16] in blood specimens obtained twice weekly in the fasting state prior to the daily aluminum injection. The content of aluminum was measured in trabecular bone samples as reported elsewhere [17]. The detailed results of serum levels of calcium, immunoreactive parathyroid hormone (iPTH), and aluminum, serial measurements of renal function, and the results of kinetic studies of aluminum are reported elsewhere [18]. The animals underwent bone biopsy prior to and after 3 to 5 weeks of parenteral aluminum loading. For the initial bone biopsy, double tetracycline labelling of bone was achieved by the oral administration of tetracycline hydrochloride (30 mg/kg) for 3 consecutive days followed by a second course of tetracycline, also of 3 days duration, after an interlabel period of 18 days duration. Because renal function declined after aluminum administration, the second bone biopsy was done earlier than planned. Thus, the double tetracycline labelling was carried out with an interval of only 6 days between the "labels" in four dogs. In the other two animals, terminated after 3 weeks of aluminum injections, only a single course of tetracycline of 2 days duration was given before the second biopsy. For all biopsies, the bone specimen was obtained 24 hr after the last dose of tetracycline.

The bone biopsy specimens were fixed for 24 hr in phosphate-buffered formalin and dehydrated by sequential passage through 70 and 90% ethanol, for 24 hr each. Final dehydration was achieved by three changes in 100% ethanol, each of 24 hr. Specimens were infiltrated with methylmethacrylate for 24 hr at room temperature and then polymerized at 35°C in a drying oven for 24 hr. The blocks were trimmed and both 5- and 10-μ sections of nondecalcified bone were made with a microtome (Model 1140, Autocut Reichert-Jung, Vienna, Austria). Five-micron sections for light microscopy were stained using the modified technique of Goldner as described in detail elsewhere [19] and mounted in Protex. For the evaluation of tetracycline fluorescent labels, unstained 10-μ sections were mounted in 10% glycerol. Five-micron sections were stained for aluminum according to the method of Maloney et al [6].

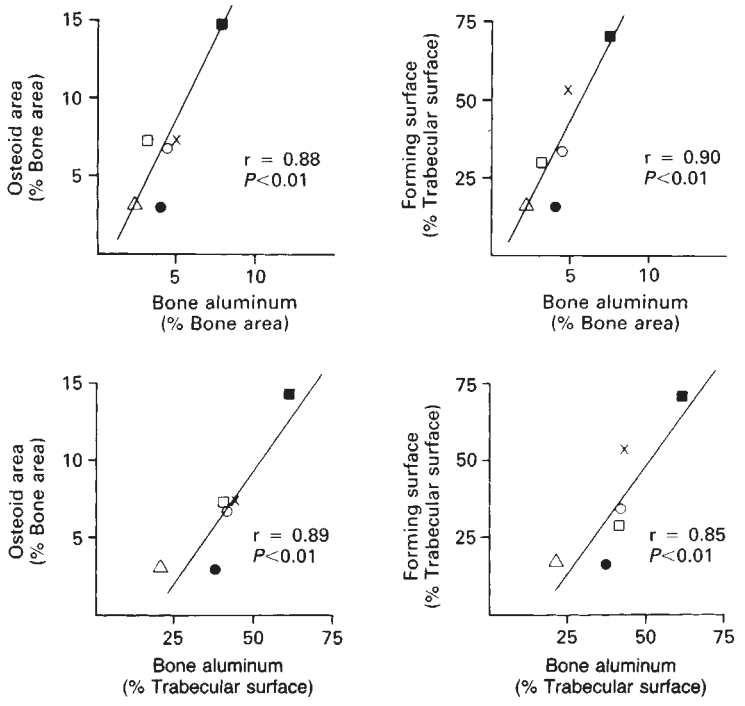

Fig. 2. *Correlations between bone aluminum content, determined by quantitative histomorphometry and expressed as both percent of trabecular surface and percent of trabecular bone area staining for aluminum, and the degree of osteoid accumulation after aluminum loading (second biopsy).* The symbols used are the same as those in Figure 1.

Light microscopy was accomplished using a Leitz microscope equipped with both tungsten and halogen light sources. Fluorescent microscopy was performed utilizing a Ploemo-Pak epiflorescent attachment with a 200-watt mercury light source. Quantitative bone histology was done using a digitizer (Summagraphics Corporation, Fairfax, Connecticut) interfaced with a series of computerized measuring programs (Model 5110, IBM, Princeton, New Jersey). Image projection was achieved by projection prism and drawing tube microscopic attachments.

The transiliac biopsy specimens were obtained from the iliac bone of all dogs from an area of 1 to 2 cm caudal to the cranial dorsal spine adjacent to the lateral cortex using the Bordier biopsy needle; this site was selected according to the report of Hardt and Jee [20]. In addition to the six dogs given aluminum, four female mongrel dogs not involved in the present investigation also underwent bone biopsy at this site. Quantitative histomorphometry was also carried out on these specimens. The variance among a minimum of six sections from each of these four biopsy specimens was analyzed for each static histological parameter measured. The mean coefficient of variation was less than 10% for all measurements with the exception of resorption surface, which was 12%.

The histologic variables for trabecular bone, determined from a minimum of three sections of bone from each biopsy, included *(1)* total bone area (%)—the area of trabecular bone, including both mineralized bone and osteoid, expressed as a percentage of the total tissue area; *(2)* osteoid area (%)—the measured area of osteoid expressed as a percent of the total bone area; *(3)* forming surface (%)—the percent of total trabecular surface covered by osteoid seams; *(4)* resorbing surface (%)—the percent of the trabecular surface characterized by the presence of scalloped bone resorbing surfaces; and *(5)* osteoid width (microns)—the mean width of surface osteoid seams, calculated by dividing the measured osteoid area, in square millimeters, by the length, in millimeters, of the osteoid seams. Bone apposition rate was calculated from the mean width of separation of the tetracycline labels divided by the time interval between labels; this is expressed in microns/day.

Unless otherwise noted, all values are expressed as the mean ± SE. Statistical analysis of the data employed Student's *t* test for paired samples and linear regression analysis.

Results

Sequential bone biopsies performed following the parenteral administration of aluminum demonstrated the development of changes consistent with histologic osteomalacia (Fig. 1). Both percent osteoid and osteoid width increased significantly from the first to the second biopsy in all dogs. The extent of trabecular bone surface covered by osteoid rose in four of six animals, but the overall change from 24.2 ± 3.4 to 37.2 ± 21.2% was not significant. However, dogs 3 and 4 exhibited substantial increments in percent-forming surface; they also showed the greatest accumulation of aluminum in bone (see below).

There was a virtual arrest of bone formation at the time of the second biopsy. Tetracycline uptake was patchy and noncontiguous at the bone-forming surfaces of all six dogs. In the four dogs with double tetracycline labeling before the second biopsy, only occasional sites of double tetracycline label were identified beneath surface osteoid seams. Because sufficient double tetracycline labeling of bone could not be identified, neither bone apposition rates nor bone formation rates could be calculated in the four dogs given double tetracycline labels after the period of aluminum administration.

The percent of trabecular resorption surface was unchanged from the first to the second biopsy (Fig. 1). However, trabecular bone area, expressed as the percent of total tissue area, decreased ($P < 0.05$).

The trabecular bone content of aluminum, determined by

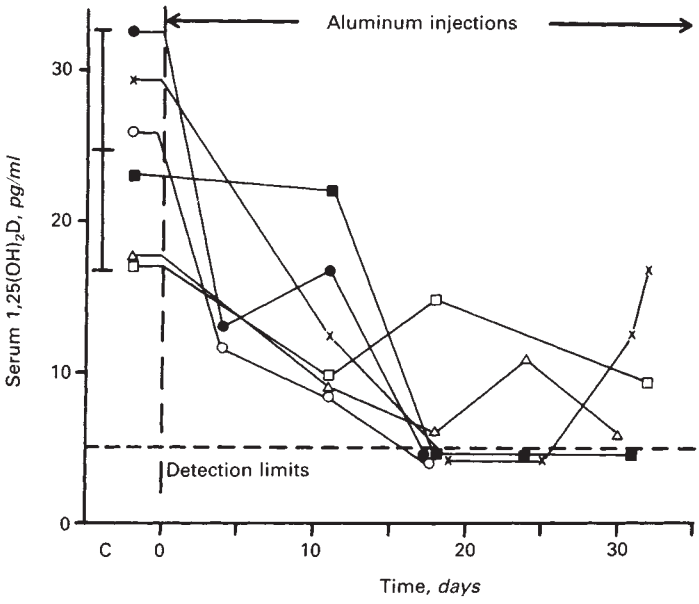

Fig. 3. *Serum levels of 1,25(OH)$_2$D* in six dogs before and after aluminum loading (1 mg Al^{+++}/kg intravenously five times per week). Control (C) values represent the mean of three to five determinations in each animal. The brackets encompass the mean ± SD for all control values. Dogs 1 and 2 received aluminum for 3 weeks only. The remaining symbols used are the same as those in Figure 1.

flameless atomic absorption spectroscopy, increased from 1.3 ± 1.6 to 94 ± 19 mg/kg dry weight following aluminum loading. Histochemical staining for aluminum revealed no aluminum in bone samples obtained prior to aluminum administration, whereas all bone specimens demonstrated positive staining at the time of second biopsy. The extent of aluminum accumulation in bone, measured histochemically, correlated with the severity of osteomalacia, as determined by both the percent of osteoid and the percent of trabecular-forming surface (Fig. 2). This was true whether bone aluminum was expressed as the percentage of trabecular surface staining positive for aluminum or as the percent of trabecular bone area which exhibited aluminum staining.

There were substantial changes in serum biochemical parameters and reductions in renal function, particularly during the later part of the course of aluminum administration; these data are reported in detail elsewhere [18]. To summarize briefly, hypercalcemia developed in five of six dogs and became marked in three animals during the few days before the studies were terminated. Also, renal function decreased in all animals, with marked changes in four. Despite the increments in serum calcium, serum iPTH levels were not suppressed in any of the dogs and actually increased above normal during aluminum administration in four animals. Serum phosphorus levels were generally not increased, except when renal function was markedly impaired in two dogs. Persistently low serum phosphorus levels, with values of 1.6 to 3.5 mg/dl, developed only in dog no. 6.

Serum levels of 25(OH)D were 45 ± 5.5 ng/ml during the control period and did not change during aluminum administration. During aluminum loading, there were substantial reductions in the serum levels of 1,25(OH)$_2$D; thus, the serum values fell from 26.8 ± 3.7 pg/ml during the control period to 12.4 ± 3.7 and 6.9 ± 1.6 pg/ml at 10 and 17 days, $P < 0.05$ and < 0.01, respectively (Fig. 3). The levels of 1,25(OH)$_2$D fell to undetectable levels (less than 5 pg/ml) in four of six dogs after 2 weeks of aluminum administration.

Discussion

The results of the present investigation clearly indicate that short-term parenteral aluminum administration can cause overt histological osteomalacia in the dog. As documented by an increase in the percentage of unmineralized bone matrix and widened osteoid seams, osteoid accumulation is a hallmark of osteomalacia. The failure to detect substantial tetracycline uptake at bone-forming surfaces at the time the second biopsy was performed also indicates both diminished bone apposition and mineralization, characteristic features of osteomalacia [21]. These findings in the dog support previous observations in the rat which suggest that aluminum loading can cause osteomalacia in trabecular bone [9, 13, 14].

Several clinical reports and histological studies of bone have indicated an association between the accumulation of aluminum in bone and dialysis-associated osteomalacia [5–10]. The results of the current investigation indicate that the retention of aluminum in bone is a factor in the pathogenesis of this syndrome. The positive correlations between the aluminum content of bone, as determined by quantitative histology, and the severity of histologic osteomalacia are analogous to results described in clinical material [5, 6, 10]. The impairment of renal function in the present dogs, described in detail elsewhere [18], was only moderate until very near the end of the study in three dogs. The histomorphological features of bone in the present study differ markedly from those of the conventional osteitis fibrosa observed in chronic renal failure [19]; thus, it is unlikely that renal failure contributed to the bone disease observed, other than by impairing the ability of the animals to excrete aluminum.

Despite the strength of the epidemiologic observations showing an association between aluminum accumulation in bone and the presence of osteomalacia, such observations could also be explained by the passive accumulation of aluminum within unmineralized osteoid. The present observations clearly exclude this as an explanation based on the documentation of normal bone histology and the absence of aluminum in bone prior to aluminum loading.

The source and time frame of aluminum exposure as well as the dosage of aluminum are clear in the present study, unlike the situation with most clinical observations of dialysis osteomalacia. The magnitude of the osseous changes observed within such a short period was unexpected. These findings indicate that relatively short-term exposure to large doses of aluminum can produce osteomalacia. Whether it is the high plasma level of aluminum present or the aluminum accumulation along bone-forming surfaces that mediates this disorder is not resolved by the present data. The preponderance of aluminum detected at the trabecular bone surfaces by histochemical stain in the present study is consistent with previous observations reported in clinical biopsy material [6, 22]. Moreover, results using the electron microprobe to localize aluminum deposition in bone confirm the surface localization of aluminum at the mineralization front of bone following aluminum exposure [5, 22]. Taken together, these observations are consistent with the local sur-

face deposition of aluminum as the pathogenic factor. The return of normal mineralization at the growth plate of rats on discontinuation of aluminum administration [9] is consistent with either the increased plasma levels of aluminum or its surface deposition in bone being responsible for the abnormality.

Bone apposition and mineralization ceased almost totally in the six dogs. Similar findings have been noted previously both in humans [7, 8] and rats loaded with aluminum [13, 14, 23]. A reduction in PTH secretion has been suggested as one explanation for these results [5, 7, 24]. The present observations clearly indicate that aluminum administration can produce osteomalacia in the presence of normal or even increased serum levels of iPTH. Moreover, observations reported elsewhere in these dogs indicate that the short-term in vivo administration of aluminum has little or no effect on PTH secretion [18]. It should be noted, however, that the present findings do not exclude the possibility that a reduction in PTH secretion may contribute to the development of "low turnover" osteomalacia in patients with prolonged exposure to aluminum.

The current observation of reduced serum levels of $1,25(OH)_2D$ in aluminum-treated dogs is of interest. This occurred in the presence of *(1)* normal levels of its precursor, 25(OH)D, *(2)* serum phosphorus values which were generally normal, and *(3)* either normal or elevated serum levels of iPTH. Renal function declined in these animals and low serum levels of $1,25(OH)_2D$ are well documented in advanced renal insufficiency [25–27]; thus, the observed changes in serum $1,25(OH)_2D$ might be explained on the basis of reduced renal function. Furthermore, the development of hypercalcemia might be expected to reduce the renal synthesis of $1,25(OH)_2D$, presumably through the suppression of PTH secretion [28]; no evidence of reduced parathyroid function was found in the current study. Moreover, the reduction in the serum levels of $1,25(OH)_2D$ occurred early in the course of the experiments and prior to the appearance of either a substantial impairment of renal function or the advent of marked hypercalcemia. Thus, the present data are most consistent with aluminum having a direct inhibitory effect on the synthesis of $1,25(OH)_2D$ by the proximal tubule. The kidneys from these dogs showed aluminum accumulation that was greater than that in most other tissues [18], a factor which might contribute to impaired biosynthesis of $1,25(OH)_2D$.

Some degree of aluminum retention is common with renal insufficiency [12]; this fact and the present observations also raise the possibility that the retention of aluminum could be a factor which contributes to the low serum levels of $1,25(OH)_2D$ in patients with advanced renal failure. This possibility seems unlikely, however, in that markedly reduced serum levels of $1,25(OH)_2D$ are observed almost uniformly in patients with endstage renal failure [25–27], regardless of whether they exhibit clinical features which suggest aluminum accumulation.

It is possible that the low serum levels of $1,25(OH)_2D$ observed could have contributed to the development of osteomalacia in the dogs in the present study; the present data do not exclude this possibility. The failure of dialysis osteomalacia to respond to treatment with $1,25(OH)_2D_3$ [29–31] makes it unlikely that altered vitamin D metabolism induced by aluminum is responsible for the osteomalacia observed clinically. It is also of interest that low serum levels of $1,25(OH)_2D$ have been noted in patients treated with long-term total parenteral nutrition [15]. Moreover, these patients may exhibit low turnover osteomalacia with aluminum accumulation in bone [32–33]. The casein hydrolysate, which had been used as a protein source in some TPN solutions, probably provided substantial quantities of parenteral aluminum to these patients [34]. The present observations suggest that not only might aluminum be responsible for impaired mineralization of bone but also raise the possibility that aluminum could be responsible for the reduced biosynthesis of $1,25(OH)_2D_3$ in these patients.

Despite the elevated serum levels of iPTH in four of six animals in the present study, the trabecular bone from these dogs showed no increase in the extent of resorptive surface. This raises the possibility that aluminum might blunt the effect of PTH to recruit additional osteoclasts. Moreover, there are data which indicate that increased aluminum levels can reduce PTH-stimulated alkaline and acid phosphatase activities in the rat calvaria studied in vitro [35].

The reduction in trabecular bone volume following aluminum administration in the present experiment was unexpected, particularly in the absence of any increase in resorptive surface of bone. This could represent sampling error as a result of variation at the site of the iliac bone selected for biopsy. However, comparisons with values for trabecular bone area determined in the first biopsy, as well as with those from four normal dogs excluded from the present study, suggest that the reduction in bone volume was not an anomalous measurement. It should be noted that the failure to identify an increase in resorptive surface does not exclude the presence of an increase in the cellular activity of osteoclasts at these surfaces. Furthermore, a reduction in bone formation could not account for the measured decline in trabecular bone volume because of the short-term nature of the present study. Thus, it is certainly possible that a substantial increase in cellular activity at bone resorptive surfaces might account for the reduction in trabecular volume observed; however, to our knowledge, there are no data to support or refute this possibility.

The current findings clearly show that the short-term administration of aluminum can lead to osteomalacia and perhaps osteopenia in dogs. Impaired release of PTH does not appear to be a factor in mediating the observed reductions in bone formation and mineralization. These latter two events appear to occur, at least partially, as a direct effect of aluminum on bone.

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References

1. Platts MM, Goods GC, Hislop JS: Composition of the domestic water supply and the incidence of fractures and encephalopathy in

patients on home dialysis. *Br Med J* 2:657–660, 1977
2. WARD MK, FEEST TG, ELLIS HA, PARKINSON IS, KERR DNS: Osteomalacic dialysis osteodystrophy: evidence for a water-borne etiological agent, probably aluminum. *Lancet* 1:841–845, 1979
3. PARKINSON IS, FEEST TG, WARD MK, FAWCETT RWP, KERR DNS: Fracturing dialysis osteodystrophy and dialysis encephalopathy: an epidemiological survey. *Lancet* 1:406–409, 1979
4. PIERIDES AM, EDWARDS WG JR, CULLU US JR, MCCALL JT, ELLIS HA: Hemodialysis encephalopathy with osteomalacic fractures and muscle weakness. *Kidney Int* 18:115–124, 1980
5. COURNOT-WITMER G, ZINGRAFF J, PLACHOT JJ, ESCAIG F, LEFEVRE R, BOUMATI P, BOURDEAU A, GARABEDIAN M, GALLE P, BOURDON R, DRUEKE T, BALSAN S: Aluminum localization in bone from hemodialyzed patients: Relationship to matrix mineralization. *Kidney Int* 20:376–385, 1981
6. MALONEY NA, OTT S, ALFREY AC, COBURN JW, SHERRARD JD: Histologic quantitation of aluminum in iliac bone from patients with renal failure. *J Lab Clin Med* 99:206–216, 1982
7. HODSMAN AB, SHERRARD DJ, ALFREY AG, OTT S, BRICKMAN AS, MILLER ML, MALONEY NA, COBURN JW: Bone aluminum and histomorphometric features of renal osteodystrophy. *J Clin Endocrinol Metab* 54:539–546, 1982
8. OTT SM, MALONEY NA, COBURN JW, ALFREY AC, SHERRARD DJ: The prevalence of aluminum in renal osteodystrophy and its relationship to response to calcitriol therapy. *N Engl J Med* 307:709–713, 1982
9. ELLIS HA, MCCARTHY JH, HERRINGTON J: Bone aluminum in hemodialysed patients and in rats injected with aluminum chloride: relationship to impaired bone mineralization. *J Clin Pathol* 32:832–844, 1979
10. COURNOT-WITMER B, ZINGRAFF J, BOURDON R, DRUEKE T, BALSAN S: Aluminum and dialysis bone disease *(letter). Lancet* 2:795–796, 1979
11. KOVALCHIK MT, KAEHNY WD, HEGG AP, JACKSON JT, ALFREY AL: Aluminum kinetics during hemodialysis. *J Lab Clin Med* 92:712–720, 1978
12. ALFREY AC, HEGG A, CRASWELL P: Metabolism and toxicity of aluminum in renal failure. *Am J Clin Nutr* 33:1509–1516, 1979
13. ROBERTSON JA, FELSENFELD AJ, HAYGOOD CC, WILSON P, CLARKE C, LLACH F: An animal model of aluminum induced osteomalacia: Role of chronic renal failure. *Kidney Int* 23:327–335, 1983
14. CHAN Y, ALFREY AC, POSEN S, LISSNER D, HILLS E, DUSTAN CR, EVANS RA: The effect of aluminum on normal and uremic rats: Tissue distribution, vitamin D metabolites and quantitative bone histology. *Calcif Tissue Int* 23:344–351, 1983
15. KLEIN GL, HORST RL, NORMAN AW, AMENT ME, SLATOPOLSKY E, COBURN JW: Reduced serum levels of 1 alpha, 25-dihydroxyvitamin D during long-term total parenteral nutrition. *Ann Intern Med* 94:638–643, 1981
16. HORST RA, LITTLEDIKE ET, RILEY JL, NAPOLI JL: Quantitation of vitamin D and its metabolites and their plasma concentrations in five species of animals. *Ann Biochem* 116:189–203, 1981
17. LEGENDRE GR, ALFREY AC: Measuring picogram amounts of aluminum in biological tissue by flameless atomic absorption analysis of a chelate. *Clin Chem* 22:53–56, 1976
18. HENRY DA, GOODMAN WG, NUDELMAN RK, DIDOMENICO ND, ALFREY AC, SLATOPOLSKY E, STANLEY TM, COBURN JW: Parenteral aluminum administration in the dog: I. Plasma kinetics, tissue levels, calcium metabolism, and parathyroid hormone. *Kidney Int* 25:362–369, 1984
19. SHERRARD DJ, BAYLINK DJ, WERGEDAL JE, MALONEY NA: Quantitative histological studies on the pathogenesis of uremic bone disease. *J Clin Endocrinol Metab* 39:119–135, 1974
20. HARDT AB, JEE WSS: Variation of trabecular bone characteristics in iliac biopsy sites of the beagle dog, in *Bone Histomorphometry-Third International Workshop*, edited by JEE WSS, PARFITT AM, Societe Nouvelle de Publications Medicoles et Dentaires, 1980, pp 492–493
21. FRAME B, PARFITT AM: Osteomalacia: Current concepts. *Ann Intern Med* 89:966–982, 1978
22. BOYCE BF, FELL GS, ELDER HY, JUNOR BJ, ELLIOT HL, GEASTALL G, FOGELMAN I, BOYLE IT: Hypercalcemic osteomalacia due to aluminum toxicity. *Lancet* 2:1009–1013, 1982
23. GOODMAN WE: Effects of aluminum on bone dynamics in the rat *(abstract). Clin Res* 31:93A, 1983
24. MORRISSEY J, ROTHSTEIN M, MAYOR G, SLATOPOLSKY E: Suppression of parathyroid hormone secretion by aluminum. *Kidney Int* 23:699–704, 1983
25. BRUMBAUGH PF, HAUSSLER DH, BRESSLER R, HAUSSLER MR: Radio-receptor assay for 1 alpha,25-hydroxyvitamin D_3. *Science* 183:1089, 1974
26. EISMAN JA, HAMSTRA AJ, KREAM BE, DELUCA HF: 1,25-dihydroxyvitamin D in biological fluids: A simplified and sensitive assay. *Science* 193:1021, 1976
27. LAMBERT PW, DEOREO PB, HOLLIS BW, FU IY, GINSBERG DJ, ROOS BA: Concurrent measurement of plasma levels of vitamin D_3 and five of its metabolites in normal, chronic renal failure, and anephric humans. *J Lab Clin Med* 98:536–548, 1981
28. HUGHES MR, HAUSSLER MR, WERGEDAL J, BAYLINK DJ, BRUMBAUGH PF: Regulation of serum 1 alpha, 25-dihydroxyvitamin D_3 by calcium and phosphate in the rat. *Science* 190:578, 1975
29. COBURN JW, BRICKMAN AS, SHERRARD DJ, SINGER FR, WONG EGC, BAYLINK DJ, NORMAN AW: Renal osteodystrophy and its relation to vitamin D: Identification of a mineralizing defect in uremia unrelated to vitamin D, in *Endocrinology of Calcium Metabolism*. Proc 6th Parathyroid Conf, Congress Series No. 421. Amsterdam, Excerpta Medica, 1977, pp 27–32
30. ALVAREZ-UDE F, FEEST TG, WARD MK, PIERIDES AM, ELLIS AA, PEART KM, SIMPSON W, WEIGHTMAN D, KERR DNS: Hemodialysis bone disease: Correlation between clinical, histologic, and other findings. *Kidney Int* 14:68–73, 1978
31. HODSMAN AB, SHERRARD DJ, WONG EGC, BRICKMAN AD, LEE DBN, ALFREY AC, SINGER FR, NORMAN AW, COBURN JW: Vitamin D-resistant osteomalacia in hemodialysis patients lacking secondary hyperparathyroidism. *Ann Intern Med* 94:629–637, 1981
32. KLEIN GL, OTT SM, ALFREY AC, SHERRARD DJ, HAZLET TK, MILLER NL, MALONEY NA, BERQUIST WE, AMENT ME, COBURN JW: Aluminum as a factor in the bone disease of long-term parenteral nutrition. *Trans Assoc Amer Physicians* 95:155–164, 1982
33. OTT SM, MALONEY NA, KLEIN GL, ALFREY AC, AMENT ME, COBURN JW, SHERRARD DJ: Aluminum is associated with low bone formation in patients on chronic parenteral nutrition. *Ann Intern Med* 98:910–913, 1983
34. KLEIN GL, ALFREY AC, MILLER NL, SHERRARD DJ, HAZLET TK, AMENT ME, COBURN JW: Aluminum loading during total parenteral nutrition. *Am J Clin Nutr* 35:1425–1429, 1982
35. LIEBERHERR M, GROSSE B, COURNOT-WITMER G, THIL CL, BALSAN S: In vitro effects of aluminum on bone phosphatases: possible interaction with bPTH and vitamin D_3 metabolites. *Calcif Tissue Int* 34:280–284, 1982